Regulation of Human Fetal and Adult Globin Genes in Mouse Erythroleukemia Cells

By B.J. Morley, C.A. Abbott, and W.G. Wood

We have examined whether transfected mouse erythroleukemia (MEL) cells can be used to examine differential expression of human γ and β-globin genes. These cells, which express only their adult globin genes, will transcribe the human adult β gene but not the fetal γ genes when they are introduced on an intact human chromosome 11 by cell fusion. However, MEL cells stably transfected with the human γ gene attached to one of the active elements (HS2) of the β-globin locus control region (LCR) readily produce γ-globin mRNAs in amounts equivalent to those seen with a comparable β gene insert. When both β and γ genes are attached to HS2, equal amounts of β and γ mRNAs are produced, irrespective of the gene order. Furthermore, when HS2 is inserted into the 5' end of a 40-kb cosmid containing the βγγ-β' genes in their normal chromosomal organization (but with the Greek HPFH-117 γ gene mutation), it directs expression of readily detectable amounts of γγ and β-globin mRNAs in MEL cells. Therefore, under these circumstances we have observed no competition between β and γ genes for expression in MEL cells. These findings suggest that MEL cells are capable of perpetuating regulatory information involved in developmental control when it is provided by an intact chromosome, but are incapable of reconstructing such information on transfected DNA.

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1355
(MEL) by cotransfecting a hamster adenine phosphoribosyl transferase (APRT) gene with constructs containing the human genes linked to one of the major hypersensitive sites on its own[12]. MEL cells are blocked at a stage in erythroid maturation before globin gene transcription but can be chemically induced to undergo full erythroid differentiation and express human globin genes at a high level. We have demonstrated that these adult erythroid cells will readily transcribe both the human fetal y-globin gene and the adult beta-globin gene. No differential expression of y and beta genes was seen when they were attached to HS2, irrespective of gene order (HS2^y-gbeta or HS2bp-gbeta), and when HS2 is attached to the y^ybeta and beta genes in their normal chromosomal organization (but carrying the Greek HPFH -117 promoter mutation), transcripts from both y genes and the beta gene were readily detectable.

**MATERIALS AND METHODS**

Construction of recombinants. Recombinant fragments were constructed in the vectors pNXKN and pNp1.1 (a gift from R.W. Jones, University of Oxford), which contain a polylinker, including two Not I restriction sites between the EcoRI and HindIII sites of pSP65 (Promega Corp, Madison, WI); pNp1.1 also has the 4.9-kb BglII fragment containing the human beta gene ligated into the BamHI site in the polylinker.

A 1.9-kb PvuIII/KpnI fragment containing HS2 from pGSE 272^ was inserted into the Apa I site of the beta gene in pNp1.1, in both the genomic and reverse orientations to give pN2p1.5 and pN2p21, respectively.

A 3.2-kb HindIII/Xmn I fragment containing the y gene from pNHYH (a gift from R.W. Jones) was cloned into the Xho I site and the 4.9-kb BglII fragment containing the human beta gene ligated into the BamHI site in the polylinker.

The recombinant pN2p17 was constructed in pNXKN by the insertion of the HS2 restriction fragment into the Xho I site and the y fragment into the Kpn I site.

All of these fragments (Fig 1) containing the genes linked to HS2 were released from the vector by digestion with Not I and gel purified before electroporation into MEL cells.

The recombinant cos ME2.5 was constructed using a cosmid containing the 40-kb Kpn I fragment encompassing the y^ybeta and beta genes but with the -117 Greek HPFH mutation in the y gene. The 1.9-kb HS2 fragment (see above) was ligated in the reverse orientation into the unique Sma I site in the cluster, 3 kb 5' of the y gene. The insert was released from the vector by an Asp 718 digest, and the DNA was phenol extracted and ethanol precipitated before electroporation.

**Isolation of stable transformants in MEL cells.** The cells used were an APRT-negative, semi-derivative of MEL line 585, cloned, and selected for a high level of inducibility before these experiments and maintained in RPMI 1640 + 15% fetal calf serum. Cells, 1 to 2 x 10^5, were cotransfected with 1.0 microgram hamster APRT (7.8-kb HindIII fragment, from pHaprt-1^10) and a 10-fold molar excess of the purified globin fragments, by electroporation at 400 V, 1,000 microfarads with laboratory-made equipment (R.W. Jones and G. Warner, University of Oxford). Selection for APRT positive cells was performed in medium containing methotrexate (10^-5 mol/L), adenine (10^-4 mol/L), thymidine (3 x 10^-2 mol/L), and hypoxanthine (10^-5 mol/L) after 24 hours. Cell colonies were counted after 5 to 7 days to give an estimate of the number of clones per pool.

Each flask was maintained in selective medium until a cell count of approximately 2 x 10^5 was attained, when half the cells were harvested for DNA isolation and the remainder were induced with 5 mmol/L hexamethylene bisacetamide (HMBA) for 3 days before harvesting for RNA analysis. Individual clones of transfected cells were obtained by plating out low numbers of cells from pools in selective medium containing 1% methylcellulose in 24-well tissue culture plates. After 1 week of incubation, single clones were picked from each well with finely drawn Pasteur pipettes and grown up in selective medium. An MEL cell hybrid containing a human X-11 translocation chromosome (HU-11) was obtained from Dr A. Skoultchi (Albert Einstein College of Medicine, New York, NY) and was maintained in Dulbecco's MEM containing methotrexate, hypoxanthine, and thymidine at the concentrations shown previously.

**DNA analysis and estimation of gene copy number.** DNA was isolated from the pooled cells and Southern blot analysis performed as described previously. For quantitation, a set of DNA controls was prepared by dilution of the construct pN2p10 in 5-ug...
aliquots of DNA isolated from MEL cells to represent two copies and eight copies per cell. These were standardized against human DNA. Five micrograms of DNA from each pool was digested with EcoRI (for \( \beta \) gene quantitation) or with BamHI/EcoRI fragment from pNpl.1 or a 0.5-kb \( \text{Pvu}^\text{II} \) fragment from pNHIHx (for \( \gamma \) gene quantitation), Southern blotted, and hybridized with probes containing the 10S-2 regions of the respective genes (a 0.9-kb BamHI/EcoRI fragment from pNPl.1 or a 0.5-kb \text{Pvu}^\text{II} \) fragment from pNHIHx). The blots were also hybridized with a 0.5-kb \( \text{Pst I} \) mouse \( \alpha \) fragment from pUCMae as a control for the level of DNA per lane. Autoradiographs, representing 18-hour to 1-week exposure aliquots of DNA isolated from MEL cells to represent and eight copies per cell. These were standardized against human quantitation), Southern blotted, and hybridized with probes cell pellets containing 2 to 100\(^5\) cells. Twenty milligrams of mouse and human hemoglobin was added to Human and mouse signals. EcoRI, and giving a protected fragment of 135 bp; (3) pG'y2.1, a DNA. Five micrograms of DNA from each pool was digested with counting. To analyze the organization of transfected DNA, a variety of digests were performed. The 4.9-kb \( \text{BglII} \) \( \beta \)-globin gene fragment, the 0.3-kb \( \text{BstUIXba I} \) fragment containing HS2, and a 2.0-kb \text{Pvu II} \) APRT\(^{13}\) gene fragment were used in addition to the probes described above.

**RNA analysis and quantitation.** Total RNA was prepared\(^{14}\) from cell pellets containing 2 to 5 \times 10\(^6\) cells. Human and mouse RNAs were detected using the qualitative RNase mapping procedure.\(^{19}\) Three plasmids containing the SP6 promoter and the \( S' \) end of the genes were used as probe templates: (1) pSPJMxS (a gift from R.W. Jones), a 103-bp \( \text{Pst I} \) \( \text{Bal I} \) fragment corresponding to the \( S' \) end of mouse \( \alpha \), linearized with \( \text{HindIII} \), and giving a protected fragment of 93 nucleotides; (2) pGP6, a 174-bp \text{BstNI} \) fragment from pBR8 containing the \( S' \) end of the human \( \beta \) gene inserted into the \( \text{Sm}a^1 \) site of pGEM I (Promega Corp.), linearized with EcoRI, and giving a protected fragment of 135 bp; (3) pG'y2.1, a 212-bp \text{BstELUMsp} I fragment from pBR\(^{8}\) containing the \( S' \) end of the human \( \gamma \) gene ligated into the \( \text{Sm}a^1 \) site of pGEM I, linearized with EcoRI, and giving a protected fragment of 142 bp. This probe does not distinguish \( \gamma \) and \( \gamma' \) gene transcripts. Therefore, for this purpose pBC'y, a 516-bp HindIII/Rsa I fragment containing the \( S' \) end of the \( \gamma \) gene ligated into the HindIII/EcoRI sites of pBCSK\(^{+}\) (Stratagene, La Jolla, CA), was used (kindly provided by Dr. M. Harvey, University of Sydney, Australia). The construct was linearized with \( \text{HindIII} \) and transcribed from the T3 promoter, giving a protected fragment of 215 bp for \( \gamma' \) and 131 bp for \( \gamma'\).

RNA probes labeled with \( \alpha\)-\( \text{P} \)-GTP were transcribed with SP6 polymerase (Amersham International, Amersham, UK) or T3 polymerase (Boehringer Mannheim, Mannheim, Germany) and approximately 1 to 10\(^6\) \text{cpm} hybridized overnight with 2 \( \mu \)g total cellular RNA. Preliminary experiments demonstrated that these hybridizations were performed in considerable probe excess. After digestion with RNases A (40 \( \mu \text{g/mL} \)) and T1 (2 \( \mu \text{g/mL} \)), the protected fragments were separated on 8\% polyacrylamide/8 mol/L urea gels and autoradiographed. Individual bands were quantitated by liquid scintillation counting after excising them from the gel, correcting for the number of G residues per protected fragment. **Analysis of globin chains.** Globin chain synthesis was measured after incubating 2 to 10\(^6\) cells with 50 \( \mu \text{Ci} \) \( ^3\)H leucine in leucine-free incubation medium\(^{15}\) made up with dialyzed fetal calf serum. Twenty milligrams of mouse and human hemoglobin was added to the lysed cells as carrier and globin was precipitated with acidified acetone and the chains separated by carboxymethyl cellulose chromatography\(^{16}\) using a combined salt and pH gradient of 0.007 mol/L Na\(_2\)HPO\(_4\) pH 6.4 to 0.028 mol/L Na\(_2\)HPO\(_4\) pH 6.8. The incorporation of \( ^3\)H leucine was measured by liquid scintillation counting.

**RESULTS**

An intact \( \beta \)-globin cluster and an LCR \( \beta \)-globin construct produce high levels of \( \beta \)-globin mRNA in MEL cells. It has previously been demonstrated that hybrid MEL cells with a human chromosome 11 derived from human adult erythroid or nonerythroid cells produce only human \( \beta \) mRNA and protein and not human \( \gamma \) mRNA.\(^{20}\) We have confirmed this observation. In a cell line containing an X-11 translocation chromosome (HU-11, obtained courtesy of Dr A. Skoulitch), the level of human \( \beta \) mRNA averaged 44\% of mouse \( \alpha \) mRNA levels (Fig 2); because there is only a single \( \beta \) gene in those cells, this is equivalent to 88\% of endogenous levels. Hybridization of a 20-fold excess of RNA to a human \( \gamma \) probe gave no detectable signal in an RNase protection assay, which, given the sensitivity of this technique, indicates that the level of \( \gamma \) gene expression is at least 500-fold less than \( \beta \) gene expression (data not shown). This confirms that MEL cells are capable of exhibiting stage-specific transcription of the human globin genes.

To provide a baseline against which to compare constructs containing the human \( \beta \) and \( \gamma \) genes, we first introduced the cosmid containing the intact \( \beta \) LCR attached to a \( \beta \)-globin gene (Fig 1) into MEL cells. On induction of hemoglobin synthesis, high-level human \( \beta \) gene expression was observed in 25 of 25 pools of cells (Table 1, Fig 2). The amount of \( \beta \) mRNA produced was quantitated relative to the endogenous mouse \( \alpha \) mRNA and ranged from 2\% to 143\%, the variability depending on the copy number (see below).

**Expression of human \( \gamma \) and \( \beta \) genes in MEL cells.** We also measured the levels of human \( \beta \)-globin mRNA produced in MEL cells stably transformed with fragments containing only one of the LCR hypersensitive sites, HS2, attached to a human \( \beta \)-globin gene (Fig 1). High-level expression was again observed in all DNA positive pools, irrespective of whether HS2 was in the normal or reverse orientation with respect to the \( \beta \) gene (Table 1, Fig 2). Quantitation of the \( \beta^{\gamma} / \alpha^{\beta} \) mRNA ratios ranged from 11% to 105% but showed even greater variation when corrected for gene copy number, revealing an inverse relationship between copy number and expression per copy (see below).

A similar result was obtained with MEL cells transfected with a fragment containing HS2 attached to the human \( \gamma \) gene in the normal orientation (Fig 1). High-level expression was observed in all DNA positive pools, irrespective of whether HS2 was in the normal or reverse orientation with respect to the \( \beta \) gene (Table 1, Fig 2). Quantitation of the \( \beta^{\gamma} / \alpha^{\beta} \) mRNA ratios being comparable to, or higher than, the \( \beta^{\gamma} / \alpha^{\beta} \) ratios seen with HS2 \( \beta \) constructs (Table 1, Fig 2) and also showing an inverse relationship between copy number and expression per copy. This result demonstrates that under these conditions, MEL cells can transcribe high levels of human \( \gamma \) mRNA, even though the gene in its normal chromosomal environment, is inactive\(^{21}\) or silenced\(^{22}\) when transferred to MEL cells.

MEL cells transfected with constructs containing HS2 linked to both the human \( \gamma \) and \( \beta \) genes arranged as either HS2 \( \gamma \) \( \beta \) or HS2 \( \beta \) \( \gamma \) (Fig 1) produced abundant amounts of both human \( \gamma \) and human \( \beta \) mRNA (Table 1, Fig 2). The average ratio of \( \gamma / \alpha^{\beta} \) mRNA was 33\% and that of \( \beta^{\gamma} / \alpha^{\beta} \) mRNA was 31\% in the HS2 \( \gamma \) \( \beta \) constructs. For HS2

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Fig 2. Expression of human β- or γ-globin RNA in induced MEL cells. (A) Representative RNase protection assay using 2 μg RNA from transfected MEL cell pools hybridized with mouse α probe and either human β or γ gene probes. The constructs used to transfect the cells are shown above the respective lanes. Full-length probes are shown at the left and controls consisted of induced MEL cell RNA and human adult or cord blood reticulocyte RNA. (B) Expression of human globin RNA after transfection of MEL cells with constructs containing HS2 attached to both human γ and β genes. Also shown (last lane) is RNA from induced MEL cells containing a human X-11 translocation chromosome (HU-11).

β\(^γ\) the relative values were 26% and 32%, respectively. Of 18 pools examined, the ratios of β\(^γ\)/β\(^H\) mRNAs produced were approximately equal (within the limits of experimental error) in all cases except one. To demonstrate that this was not the result of averaging the values from several different clones in each pool, individual cells from several pools were cloned in methylcellulose and expanded and induced for analysis. Each clone also produced approximately equal amounts of human γ\(^γ\) and β mRNA, irrespective of the gene order. The exception to this pattern was a single pool containing HS2 β \(^γ\) in which the transfected DNA remained unrearranged but in which only trace amounts of γ\(^γ\)

<table>
<thead>
<tr>
<th>Construct</th>
<th>No. of Pools</th>
<th>No. of Clones per Pool Mean (range)</th>
<th>β(^H)/α(^M) mRNA(%)</th>
<th>γ(^H)/α(^M) mRNA(%)</th>
<th>Copy No.</th>
<th>% Human RNA per Copy Range</th>
<th>Estimated % Human RNA per Single Copy</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS432β1</td>
<td>25</td>
<td>3 (1-9)</td>
<td>2-143 36</td>
<td>—</td>
<td>0.3-19</td>
<td>3 1-136</td>
<td>91</td>
</tr>
<tr>
<td>HS2β</td>
<td>9</td>
<td>17 (11-25)</td>
<td>16-77 50</td>
<td>—</td>
<td>0.2-8</td>
<td>2 2-195</td>
<td>61</td>
</tr>
<tr>
<td>HS2β+</td>
<td>12</td>
<td>2 (1-3)</td>
<td>11-105 60</td>
<td>—</td>
<td>0.6-34</td>
<td>8 1-56</td>
<td>47</td>
</tr>
<tr>
<td>HS2 (^γ)</td>
<td>13</td>
<td>6 (1-10)</td>
<td>—</td>
<td>53-220 102</td>
<td>6-72</td>
<td>12 2-26</td>
<td>64</td>
</tr>
<tr>
<td>HS2 (^γ)β</td>
<td>11</td>
<td>3 (1-160)</td>
<td>1-106 31</td>
<td>1-108 33</td>
<td>&lt;0.5-36</td>
<td>5 3-58</td>
<td>50</td>
</tr>
<tr>
<td>HS2 β(^γ)</td>
<td>7</td>
<td>3 (2-5)</td>
<td>3-90 32</td>
<td>4-77 26</td>
<td>0.5-16</td>
<td>4 3-30</td>
<td>27</td>
</tr>
</tbody>
</table>

*Estimated from graph of expression per copy versus copy number, as in Fig 5.
†HS2 in inverse orientation.
mRNA were detected while the β mRNA level was similar to that in the other pools. The combined levels of human β and γ mRNA in these stable transformants averaged 65% of the endogenous mouse α mRNA levels, similar to the values obtained with either γ or β gene alone with HS2. Furthermore, when globin chain synthesis was measured in these cells, approximately equal amounts of human γ and β chains were demonstrated, in amounts approximately equal to the endogenous mouse β-chain synthesis and averaging about 50% of the mouse α-chain production (Fig 3).

We also cotransfected MEL cells with APRT and a construct containing HS2 inserted into the 5' end of the 40-kb *Kpn* I fragment containing the γ, γ, δ, and β genes in their normal genomic organization (Fig 1). The γ gene in this construct contained the G to A substitution at position −117 in the promoter, a change that produces an HPFH phenotype in vivo. Analysis of the RNA from induced cells resulted in readily detectable expression of the human globin genes in 20 of 20 pools (Fig 4A). The overall levels of expression (Table 2) relative to αM were lower than those seen with HS2 γ, β, and HS2 γ, β constructs, though the average copy number of the exogenous DNA per cell was high (18 to 63 copies). In each of the 20 pools, all three human genes were expressed. The relative proportions of α, γ, and β mRNA were approximately 1:2:1 (Table 2).

**Relationship of gene copy number to exogenous RNA production.** In previous studies of the β-globin cosmid or HS2 constructs transfected into MEL cells, the level of RNA production was reported to be directly related to the copy number of the transfected genes. However, in our studies the level of human mRNA detectable in pools containing an average of one or more copies per cell varied over only a narrow range despite wide differences in copy number. When mRNA output per gene copy is plotted against copy number, an inverse correlation is obtained (Fig 5), suggesting that at high copy numbers, there is a reduction in the number of transcripts from each copy that posttranscriptional processes limit the accumulation of globin mRNA. This analysis was performed on pools of transfected cells which, as a result of the cotransfection procedure, probably comprise mixtures of cells with and without the exogenous globin genes. Therefore, no conclusions can be drawn about the exact nature of this inverse relationship, which would require an analysis of a large number of individual clones containing variable numbers of the integrated genes. Nevertheless, the fact that such a relationship can be observed in pools suggests that extreme caution should be exercised in drawing conclusions about the level of expression per gene copy.

**Inducibility and stability of transfected genes.** In keeping with previous results, the constructs used in this study showed inducible expression in parallel with the endogenous mouse globin genes on treatment of the transfected cells with HMBA (data not shown). In several cases the transfected human genes showed high levels of expression in the uninduced cells, with only a small increase (1.5- to 4-fold) after induction. In these cells we presume that position effects allow high levels of transcription even though the endogenous genes are transcriptionally silent.

The stability of the transfected cells was examined by maintaining pools and individual clones of cells transfected with HS2 γβ and HS2 γ, β in continuous culture for 9 months. RNA analysis at frequent time points throughout this period showed no differences in the proportions of human γγ and β mRNA (data not shown).

**Organization of the integrated exogenous genes.** Analysis of the DNA from transfected cells by Southern blotting demonstrated that in almost all the pools (51 of 52) and clones (10 of 10) examined, tandem head-to-tail repeats could be demonstrated. This was the case even in pools with a low overall copy number. Bands corresponding to head-to-head and tail-to-tail repeats were also seen in many samples, although at much lower levels. Analysis of the cotransfected APRT gene showed a low copy number (~1) in all cases, with no tandem repeats. In the majority of cases the gene appeared to be integrated at a separate site from the globin genes. Thus, by using cotransfection, the potential problem of selecting sites of integration on the expression of a linked selectable marker has been obviated.

**DISCUSSION**

The results reported here confirm the importance of elements of the β LCR for high-level expression of the β-like genes and confirm that one of these elements, HS2 at −10.7 kb, (previously known as HSII; or HS 3') can, to a large degree, substitute for the whole of the LCR complex in allowing high-level expression. Previous work has demonstrated that γ genes without an LCR can be expressed after transfection into MEL cells but only at a very low level and are presumably dependent on the site of integration; contradictory results were obtained as regards appropriate regulation of the gene on induction. We have

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**Figure 3.** Globin chain synthesis in a clone of MEL cells transfected with HS2γβ. Cells were labeled for 4 hours with 3H leucine, 72 hours after induction with HMBA. γ, β, human γ and β chains; α, α, mouse α and β chains.

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now demonstrated that there is no differential effect of HS2 on γ and β gene expression in MEL cells; HS2 attached to the αγ gene alone produces mRNA expression levels as high, if not higher than when it is attached to the β gene alone. Furthermore, when constructs containing HS2 upstream of both genes are expressed in stable MEL cell transformants, both genes are expressed in high, approximately equal amounts, irrespective of the order of the globin genes. This high-level expression can occur, in some cases, before induction of the endogenous genes, which suggests that all the trans-acting factors necessary for globin gene transcription are present before induction and that there must be additional mechanisms responsible for the fact that before induction the endogenous genes are repressed while the activity of the exogenous genes depends on their site of integration.

With the construct containing HS2 inserted at the 5′ end of the 40-kb fragment containing the 0γγ y−117 δ and β genes in their normal genomic organization, expression of the 0γ, αγ, and β genes was readily detectable in all pools in an approximate ratio of 1:2:1. The increased αγ gene expression may be due, in part, to the fact that the αγ gene in this construct contains a known nondeletion HPFH mutation at position −117. In vivo, the relative expression of the three genes is 0.1:1:2; and, transfer of a chromosome 11 containing this mutated αγ gene to MEL cells resulted in an expression pattern in which αγ chain synthesis greatly exceeded β and αγ-chain production. While it will be necessary to confirm the expression results from the HS2 γγ y−117 β construct using a normal cosmid, we would stress that in MEL cells, the αγγ and β genes are expressed at levels much more equal to each other than they are in vivo or after chromosome transfer.

Previous studies in transgenic mice or transfected MEL cells have produced variable results with respect to the copy number dependent levels of human β-globin gene expression. It is not yet clear to what degree copy number dependence may be influenced by whether an intact LCR, a microlocus containing the four hypersensitive sites, or just HS2 on its own has been used in these experiments. In our series, an inverse correlation between expression per gene copy and copy number was clearly demonstrated, a relationship that we also found in MEL cells transfected with constructs containing the major upstream regulatory region of the α-globin locus linked to an α-globin gene. It may be that while there is copy number dependent expression at the low end of the range, it is lost or even reversed at high copy numbers. It remains to be seen whether this effect is responsible for the lower levels of expression seen with the HS2 γγ y−117 β construct where consistently high copy numbers were observed.

Our results in MEL cells show no evidence of competition between the γ and β genes for expression. These results are significantly different from those obtained when related constructs are introduced into transgenic mice. Transgenic mice containing the human γ or β genes, with no LCR elements attached, show low-level, tissue-specific expression with developmental regulation. Attachment

### Table 2. RNA Analysis of 20 Pools of MEL Cells Transfected With HS2 γγ y−117 β

<table>
<thead>
<tr>
<th>No. of Clones per Pool</th>
<th>αγγ mRNA %</th>
<th>αβθ mRNA %</th>
<th>βθθ mRNA %</th>
<th>γβθ mRNA %</th>
<th>γγγ mRNA %</th>
<th>Copy No.</th>
<th>% Human mRNA per Gene Copy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>3.5 ± 1.8</td>
<td>7.5 ± 4.9</td>
<td>2.9 ± 1.3</td>
<td>3.8 ± 1.9</td>
<td>0.50 ± 0.11</td>
<td>46 ± 11</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Range</td>
<td>5-14</td>
<td>0.4-7.5</td>
<td>0.8-22.7</td>
<td>0.8-6.5</td>
<td>0.5-1.6</td>
<td>16-63</td>
<td>0.2-1.6</td>
</tr>
</tbody>
</table>
of various elements of the LCR to either gene confers high-level expression but developmental stage specificity is lost.\(^{10,11,13,40}\) However, developmental regulation is restored when the intact LCR or the four hypersensitive sites are transferred to mice along with a fragment containing the \(0.9\gamma \gamma \beta \beta\) region of the human cluster.\(^{10,11}\) This has led to the suggestion that competition between \(\gamma\) and \(\beta\) genes is a necessary facet of developmental regulation.

The lack of competition between the \(\gamma\) and \(\beta\) genes seen in our experiments after transfection of MEL cells could be due to the use of HS2 alone in place of the intact LCR or an abbreviated construct containing all four HSs. It is clearly sufficient to allow high-level expression of both genes, and while it might have been imagined that a single site is more likely to be selective than multiple sites, it is possible that a complex interaction between the various hypersensitive sites and the gene promoters is necessary for proper regulation.

It is also possible that the relative spacing of the LCR and the \(\gamma\) and \(\beta\) globin genes is important for their regulation, although this is made less likely by the fact that chromosomes with only a single \(\gamma\) gene\(^{41}\) or with up to five \(\gamma\) genes show normal developmental switching.\(^{45}\) Certainly, there was little difference in the expression patterns of the HS2\(^{2}\gamma \beta\) and HS2\(^{2}\gamma \gamma \gamma \gamma \gamma \gamma \beta \beta\) constructs to suggest that the organization of the various components in the system may be critical to their expression.

Both of these explanations are made further unlikely by the fact that both the HS2\(^{2}\gamma \beta\) and HS2\(^{2}\gamma \gamma \gamma \gamma \beta \beta\) constructs show developmental regulation in transgenic mice (unpublished observation). A major difference between using transgenic mice and transected cells for examining globin gene expression is that in the former case, the injected DNA passes through a normal developmental history before expression of the genes in mature erythroid cells, while in the latter system the introduced DNA, although integrated into the mouse genome, can only form chromatin with the protein factors available in the MEL cell. It could be that the important trans-acting factors that determine stage specificity are not those present at the time of globin gene transcription but those present at an earlier stage in the history of an erythroid cell (eg, BFU-E, pluripotent stem cell), which could determine which gene will later be available for expression. Therefore, MEL cells might be permissive for expression of any transfected DNA containing appropriate erythroid cell signals. If this were so, it would explain why both \(\gamma\) and \(\beta\) genes are expressed in MEL cells after transfection but show developmental regulation in transgenic mice.

This explanation would also be consistent with the observation that MEL cell-human chromosome 11 hybrids ultimately express only the adult \(\beta\) gene;\(^{21,43,45}\) except when the chromosome 11 is derived from HPFH patients when the \(\gamma\) genes are expressed in the hybrid cells.\(^{30}\) These hybrid cells receive an intact chromosome, with its associated proteins, which has been through a normal developmental history. Therefore, MEL cells may be capable of perpetuating regulatory information associated with the transferred chromosome but be incapable of reconstructing such information on naked DNA. If this interpretation is correct, the important events in the developmental regulation of the globin genes may occur not in the recognizable erythroid cells but in an earlier precursor.

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